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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/827,854	04/05/2001	Vassilis I. Zannis	07180/004003	6635

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EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1636	15

DATE MAILED: 08/20/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/827,854	ZANNIS ET AL.	
	Examiner	Art Unit	
	Quang Nguyen, Ph.D.	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *09 January 2003 and 23 May 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *30-47 and 50-62* is/are pending in the application.

4a) Of the above claim(s) *32,35,38-42 and 45* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *30,31,33,34,36,37,43,44,46,47 and 50-62* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6,7,9* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Applicants' amendments filed on 1/9/03 and on 5/23/03 have been entered as Paper Nos. 11 and 14, respectively.

Applicant's election without traverse of Group I (claims 30-47) in Paper No. 11 is acknowledged. Applicants further elected without traverse (a) the species of SEQ ID NO:15 (apoE3) and (b) the species of adenoviral vector.

Claims 30-47 and 50-62 are pending in the present application.

Claims 32, 35, 38-42 and 45 are withdrawn from further consideration because they are drawn to non-elected species.

Accordingly, claim 30-31, 33-34, 36-37, 43-44, 46-47 and 50-62 are examined on the merits herein.

Priority

It is noted that this application appears to claim subject matter disclosed in prior Application No. 09/679,088 filed 10/04/2000 and in prior Application No. 09/544,386 filed on 4/06/2000. A reference to the prior applications must be inserted as the first sentence of the specification of this application or in an application data sheet (37 CFR 1.76), if applicant intends to rely on the filing date of the prior application under 35 U.S.C. 119(e) or 120. See 37 CFR 1.78(a). For benefit claims under 35 U.S.C. 120, the reference must include the relationship (i.e., continuation, divisional, or continuation-in-part) of all nonprovisional applications. Also, the current status of all nonprovisional parent applications referenced should be included. Examiner notes that the reference

to the prior applications does not appear as the first sentence of the specification of this application. Appropriate correction is required.

Claim Objections

Claim 46 is objected to because the term "LDL" should be spelled out in full at the first occurrence of the term. Appropriate correction is required.

Claims 56-62 are objected to because they contain the non-elected species (e.g., SEQ ID NOs. 14, 16-19). Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

With respect to the elected invention and species, claims 30-31, 33-34, 36-37, 43-44, 46-47 and 50-62 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of lowering cholesterol in a mammal that lacks an endogenous normally functioning apoE gene, said method comprises administering intravascularly into said mammal a recombinant replication defective adenovirus containing a nucleic acid encoding a polypeptide of between 150 and 299 amino acids that has an amino acid sequence at least 80% identical to that of the corresponding region of amino acids 1 to 299 of a mature, native, human apoE3 polypeptide, wherein said polypeptide is expressed and the total serum cholesterol level

in said mammal is lowered without inducing hypertriglyceride, does not reasonably provide enablement for a method of lowering cholesterol, delaying the onset of atherosclerosis, or regressing atherosclerosis in any mammal without inducing hypertriglyceridemia by administering via any route of delivery or expressing in any tissues in said mammal a recombinant adenovirus containing a nucleic acid encoding a polypeptide of between 150 and 299 amino acids that has an amino acid sequence at least 80% identical to that of the corresponding region of amino acids 1 to 299 of a mature, native, human apoE3 polypeptide. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The instant claims are drawn to a method of lowering cholesterol, delaying the onset of atherosclerosis, or regressing atherosclerosis in a mammal without inducing hypertriglyceridemia, said method comprising administering to or expressing in said mammal a nucleic acid encoding a polypeptide of between 150 and 299 amino acids that has an amino acid sequence at least 80% identical to that of the corresponding region of amino acids 1 to 299 of a mature, native, human apoE polypeptide, and that,

when administered to or expressed in a mammal lowers the total serum cholesterol level without inducing hypertriglyceridemia; and wherein the human apoE polypeptide is human apoE3 having SEQ ID NO:15 and the nucleic acid is contained in an adenoviral vector as the elected species.

The specification teaches by exemplification showing the construction of recombinant adenoviruses expressing apoE4 and various truncated forms of apoE4 (e.g., apoE4-185, apoE4-202, apoE4-229, EpoE4-259). In an apoE-deficient mouse model, the recombinant adenoviruses were injected intravenously through the tail vein and the effects of full-length apoE4 and its various truncated forms on cholesterol and triglyceride homeostasis were evaluated. Applicants demonstrated that an insignificant reduction in the mouse cholesterol level and a severely induced hypertriglyceridemia were observed in apoE-deficient mice treated with full-length apoE4-adenovirus, whereas reduced levels of cholesterol without the induction of hypertriglyceridemia were obtained in animals treated with recombinant adenoviruses expressing the various truncated forms of apoE4. Applicants further demonstrated that overexpression of either apoE3 or apoE4 is sufficient to induce combined hyperlipedimia (high cholesterol and triglyceride levels) in normal C57BL6 mice, whereas an overexpression of apoE4-202 has no detectable effect on triglyceride levels of the C57BL6 mice.

The above evidence has been noted and considered. However, the evidence is not reasonably extrapolated to the instant broadly claimed invention for the following reasons.

(a) The breadth of the claims. With respect to the elected species, the claims encompass a method for a method of lowering cholesterol, delaying the onset of atherosclerosis, or regressing atherosclerosis in any mammal (e.g., normal, a mammal lacking an endogenous normally functioning apoE gene, a mammal lacking an endogenous normally functioning LDL receptor) without inducing hypertriglyceridemia, said method comprises any route of delivering a recombinant adenovirus containing a nucleic acid encoding a polypeptide of between 150 and 299 amino acids that has an amino acid sequence at least 80% identical to that of the corresponding region of amino acids 1 to 299 of a mature, native, human apoE3 (SEQ ID NO: 15) into said mammal, and whereby the total serum cholesterol level is lowered without inducing hypertriglyceridemia.

(b) The state and the unpredictability of the art. The nature of the instant claims falls within the realm of gene therapy. At the effective filing date of the present application, the state of the gene therapy art remains unpredictable particularly for the attainment of the desired prophylactic and/or therapeutic effects, for this instance a delay on the onset of atherosclerosis in any mammal, including a normal mammal, as well as a regression of atherosclerosis, as evidenced by the reviews of Verma et al. (Nature 389:239-242, 1997; IDS), Dang et al. (Clin. Cancer Res. 5:471-474, 1999) and Romano et al. (Stem Cells 18:19-39, 2000). Dang et al. stated "Although significant progress has been achieved in our understanding of the limitations of gene therapy by suboptimal vectors, host immunological responses to the vectors, and the lack of long term stable expression, the major challenge that limits clinical translation remains in

achieving efficient gene delivery to target tissues" (page 474, col. 2, last paragraph). Romano et al. stated "The potential therapeutic applications of gene transfer technology are enormous. However, the effectiveness of gene therapy programs is still questioned" (see abstract), and "Despite the latest progress reported in the area of vector design, research strategies still have to tackle critically important issues, such as further improvement of gene transfer technology, especially for *in vivo* gene delivery applications, regulation and control of the transgene expression post-cell transduction, and a variety of complex safety matters. These three main issues are to some extent intertwined and pose severe limitations on the applications of gene transfer technology in therapy" (page 21, col. 1, first paragraph). Kypreos et al. (FASEB J. 15 :1598-1600, 2001) stated "One major parameter in successful gene therapy approaches is gene dosage and expression levels....The inability of the truncated apoE forms that lack all or part of the carboxyl-terminal 260-299 region to induce hypertriglyceridemia, coupled with their intact ability to clear cholesterol, makes them attractive candidates in future gene therapy applications to correct remnant removal disorders" (page 1600, col. 2, last paragraph).

(c) The amount of direction or guidance presented. Apart from exemplification using the apoE-deficient mouse model, the instant specification fails to provide sufficient guidance for a skilled artisan on how to lower cholesterol levels, delay the onset of atherosclerosis or regress atherosclerosis without inducing hypertriglyceridemia in any mammal using a recombinant adenovirus containing a nucleic acid encoding a polypeptide of between 150 and 299 amino acids that has an

amino sequence at least 80% identical to that of the corresponding region of amino acids 1 to 299 of a mature, native, human apoE3 polypeptide as broadly claimed. On the contrary, the specification teaches specifically that overexpression of either apoE3 or apoE4 in normal C57BL6 mice was sufficient to induce combined hyperlipidemia (high cholesterol and triglyceride levels), and the overexpression of apoE4-202 in normal mice increased the cholesterol levels of normal mice (see Fig. 16A). Additionally, Dijk et al. (J. Lipid Res. 40:336-344, 1999, IDS) demonstrated that in LDL receptor-deficient mice both low and high expression of apoE3 via adenovirus-mediated gene transfer did not result in a reduction of hypercholesterolemina, and severe hypertriglyceridemia was always induced (see abstract and Fig. 1). Dijk et al. further noted that for efficient clearance the non-LDL receptor-mediated pathway requires a higher level of apoE expression as compared to the LDL receptor, but is more sensitive to an apoE-induced increase in VLDL production and inhibition of VLDL-triglyceride lipolysis.

There is also no evidence of record indicating that any prophylactic effects (e.g., delaying the onset of atherosclerosis in any mammal) and/or a regression of any established atherosclerosis in a mammal have been obtained using the method as claimed, particularly the lack of a stable *in vivo* transgene expression has been known in the art (see the aforementioned reviews). The *in vivo* plasma levels of exogenous apoE using adenovirus-mediated gene transfer declined rapidly after 14 days (Dijk et al., see Fig. 1E&F; Tsukamoto et al., J. Clin. Invest. 100, 107-114, 1997, IDS, see page 109, col. 2, first paragraph). It should be emphasized that gene dosage and expression

levels of apoE are critical for the attainment of prophylactic and/or therapeutic effects without the induction of hypertriglyceridemia as contemplated by Applicants. Mahley et al. (Curr. Opin. Lipidology 10:207-217, 1999, IDS) stated "It may well be that a rather narrow range of apoE levels is required to maintain normal plasma lipid levels. If the apoE level is too high, the lipid raising effects of inhibited lipolysis and increased VLDL production can offset the benefits of increased lipoprotein clearance rate. If the apoE levels are too low (the most dramatic situation being apoE deficiency), clearance is impaired, which can lead to hyperlipidemia. If there is a 'beneficial' or 'optimal' range for apoE levels, then controlling plasma apoE levels may represent a new therapeutic target. The question arises whether regulation of apoE synthesis is possible and whether it is important for modulating lipoprotein levels" (page 212, col. 1, first paragraph). On the same issue, it is unclear on the basis of the present disclosure how a skilled artisan can utilize a replication competent recombinant adenovirus expressing apoE3 to achieve the optimal range of apoE3 level to yield the desired results, e.g., lowering the cholesterol level without induction of hypertriglyceridemia, particularly knowing the propensity of replication competent recombinant adenovirus to infect numerous cell types in the treated mammal and there is an increased probability of overexpression apoE3 in tissues and/or organs of the treated mammal, particularly the liver, which would lead to the inhibition of lipolysis and increased VLDL production.

With respect to any route of administering a recombinant adenovirus containing a nucleic acid encoding an apoE3 polypeptide of the present invention into a mammal, apart from the intravenous delivery route demonstrated in the apoE deficient mouse

model, the instant specification fails to provide sufficient guidance for a skilled artisan on how to obtain an effective level of exogenous plasma apoE3 to yield the desired prophylactic and/or therapeutic effects without the induction of hypertriglyceridemia in a mammal by delivering the recombinant adenovirus to any non-hepatic tissues (e.g., skin, brain, muscle) or by any route of delivery for liver targeting. Vector targeting *in vivo* to desired tissues or organs (for this instance to the liver) continues to be unpredictable and inefficient. This is supported by numerous teachings available in the art as well as the aforementioned review articles (see Dang et al., Verma et al., Romano et al.).

Moreover, apart from the unpredictability of gene therapy for obtaining therapeutic effects, the physiological art is also recognized as unpredictable (MPEP 2164.03). As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues discussed above, the unpredictability of the gene therapy as well as physiological art in general, and the breadth of the instant claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 30-31, 33-34, 37, 43-44, 47, 50-53 are rejected under 35 U.S.C. 102(b) as being anticipated by Tsukamoto et al. (J. Clin. Invest. 100:107-114, 1997; IDS) as evidenced by Breslow et al. (J. Biol. Chem. 257:14639-14641, 1982).

Tsukamoto et al. teach the preparation of replication-defective recombinant adenoviruses expressing human apoE3, and 1.5 X 10^{11} particles of the recombinant viral particles were injected intravenously into male apoE-deficient mice that were placed on a Western diet (see Methods section on page 108). In this study, the human apo3 cDNA was obtained from Dr. J. Smith and Dr. J. Breslow from The Rockefeller University, said sequence encodes for a mature human apo3 polypeptide of 299 amino acid residues as evidenced by Breslow et al. (see abstract). Tsukamoto et al. further note that it is well known that more than 90% of recombinant adenovirus infused intravenously is delivered to the liver (page 110, col. 1, first paragraph), and that apoE3 mRNA was detected in the liver of treated mice. Tsukamoto et al. also teach that plasma total cholesterol levels in mice injected with the apoE3 virus decreased dramatically after injection (194 mg/dl 3 day after injection vs 1,384 mg/dl before injection), and a small but significant decrease in plasma triglyceride level was also observed (see Figure 8). It is also interesting to note that mice injected with apoE2

virus had a significant increase in plasma triglyceride level (hypertriglyceridemia) unlike the situations of mice injected with either apoE3 or apoE4 virus.

Accordingly, the method of Tsukamoto et al. teach every limitation of the instant claims and therefore Tsukamoto et al. anticipate the instant claims.

Claims 30-31, 33-34, 36-37, 43-44, 47, 50-53 are rejected under 35 U.S.C. 102(b) as being anticipated by Kashyap et al. (J. Clin. Invest. 96:1612-1620, 1995) as evidenced by Breslow et al. (J. Biol. Chem. 257:14639-14641, 1982).

Kashyap et al. teach the preparation of a recombinant defective adenovirus expressing a full-length mature human apoE3 polypeptide of 299 amino acids as evidenced by Breslow et al. (see abstract). Kaqshyap et al. also teach to infuse intravenously the recombinant adenovirus into apoE-deficient mice (see abstract and Methods section). The treatment results in normalization of the lipid and lipoprotein profile with markedly decreased total cholesterol, VLDL, IDL, and LDL, as well as increased HDL (all of which are indication of the absence of hypertriglyceridemia). The expression of the transgene was predominant found in the liver (see Figure 2). Kashvap et al. further teach that 4-month old apoE-deficient mice infused with rAdv.apoE had mean aortic lesion areas that were significantly smaller than animals infused with controlled vector or control apoE-deficient mice (see page 1616, last paragraph of col. 2 continues to first paragraph of col. 1 on page 1618, and Fig. 5). Due to the ability of adenovirus vector to transduce a wide variety cells, intravenous infusion

would inherently result in the transduction of cells at aortic lesion areas in apoE-deficient mice even though liver is a major target site.

Accordingly, the method of Kashyap et al. et al. teach every limitation of the instant claims and therefore Kashyap et al. et al. anticipate the instant claims.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gerald Leffers, Jr., Ph.D., may be reached at (703) 305-6232, or SPE, Remy Yucel, Ph.D., at (703) 305-1998.

Quang Nguyen, Ph.D.

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